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# (54) CRYSTALLINE ANTHRACYCLINE ANTIBIOTIC AND PROCESS FOR PRODUCING THE SAME

(57) Disclosed are a crystalline form of anthracycline antibiotic having specific characteristic 2θ values as measured by the X-ray diffraction method, and a process for producing the crystalline form. This process comprises the step of crystallization involving the combined use of a specific poor solvent for the antibiotic and a good solvent therefor. This crystalline form has excellent chemical and physical properties.

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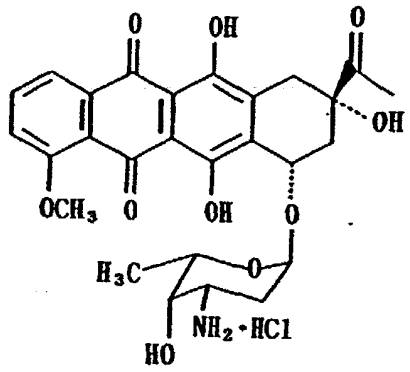
## Description

### Technical Field

[0001] This invention relates to novel crystalline forms of an anthracycline antibiotic, particularly daunomycin (also known as daunorubicin), and a process for producing the same.

### Background Art

[0002] Daunomycin (also known as daunorubicin; hereinafter abbreviated as DM), which is an anthracycline antibiotic represented by the following formula (I)



(I)

is known to be obtained from a culture medium of an actinomycete, and has a wide anticancer spectrum against experimental animal tumors. As a matter of fact, DM is being widely used as a chemotherapeutic agent for cancer in clinical applications.

[0003] However, the currently available bulk form of DM (DM hydrochloride) is an amorphous powder or a solid which is tentatively classified as crystalline but has high hygroscopicity and poor stability. From the viewpoint of the preparation of DM into medicines, the physical and chemical properties of not only its final bulk powder but also its intermediate products have a great significance. For example, poor chemical stability requires great caution in storage, and high hygroscopicity makes its handling difficult. Moreover, with consideration for its use as a drug, any residual solvent may constitute a fatal shortcoming.

[0004] Accordingly, an object of the present invention is to provide a solid product of DM hydrochloride having excellent chemical stability and, preferably, further having low hygroscopicity and an allowable residual solvent content.

### Disclosure of the Invention

[0005] The present inventors made repeated investigations with a view to solving the above-described problems and have now found that the crystallization of DM hydrochloride by using a certain solvent system yields a specific crystalline form of DM hydrochloride having excellent chemical stability and, in some instances, this crystalline form also has low hygroscopicity and can solve the problem with residual solvent.

[0006] Thus, according to the present invention, there is provided a crystalline form of DM hydrochloride having at least characteristic 2θ values (in degrees) of 6.18, 7.88, 9.82, 11.60, 13.30, 15.80, 20.88 and 23.12 as measured by the X-ray powder diffraction method.

[0007] According to the present invention, there is also provided a process for producing the aforesaid crystalline form of DM hydrochloride from a solution containing DM hydrochloride, the process comprising the steps of preparing the aforesaid solution by using a solvent system composed of a poor solvent for the antibiotic and a good solvent which is miscible with the poor solvent and capable of dissolving the antibiotic; and subjecting the solution so prepared to a crystallization treatment.

## Brief Description of the Drawing

[0008]

FIG. 1 is a chart showing the results of X-ray powder diffraction analysis of DM hydrochloride powders and various crystalline forms of DM hydrochloride. In this chart, b) shows the result of X-ray powder diffraction analysis of a crystalline form of DM hydrochloride in accordance with the present invention; a), d), e) and f) show the results of X-ray powder diffraction analysis of amorphous DM hydrochloride powders (comparative powders); and c), g) and h) show the results of X-ray powder diffraction analysis of solid forms of DM hydrochloride which are regarded as crystalline but do not show the properties of the crystalline form in accordance with the present invention (comparative crystalline forms).

## Specific Description of the Invention

[0009] Specifically, the crystalline form of DM hydrochloride in accordance with the present invention are characterized by having at least characteristic 2θ values (in degrees) of 6.18, 7.88, 9.82, 11.60, 13.30, 15.80, 20.88 and 23.12 as measured by the X-ray powder diffraction method (the Debye-Scherrer method) [see b) in FIG. 1]. The term "crystalline form" as used herein means a single crystal or a mass of such crystals, and the aforesaid results of X-ray powder diffraction analysis are those obtained from such masses.

[0010] The crystalline form in accordance with the present invention are clearly distinguished from amorphous powders [corresponding to those shown as a), d), e) and f) in FIG. 1] and solid forms tentatively regarded as crystalline [corresponding to those shown as c), g) and h) in FIG. 1]. Moreover, as will be described later, the crystalline form b) have very excellent properties from the viewpoints of hygroscopicity, residual solvent and chemical stability.

[0011] Generally and not by way of limitation, the process for producing the aforesaid crystalline form in accordance with the present invention comprises the steps of preparing a solution by dissolving a DM hydrochloride powder having a relative purity of greater than 90% in a solvent capable of dissolving the DM; and crystallizing the DM by adding to the solution a solvent which is miscible with the aforesaid solvent but is a poor solvent for DM.

[0012] It is important to use a solvent containing at least 1-butanol as the aforesaid poor solvent. Typical examples of such solvents include 1-butanol alone and solvent mixtures composed of 1-butanol and other organic solvents (e.g., acetone, hexane and diisopropyl ether). On the other hand, as the solvent capable of dissolving DM, there may be used any solvent that can dissolve DM, is miscible the aforesaid poor solvent, and hence suits the purpose of the present invention. Typical examples of such solvents include, but are not limited to, water, methanol, ethanol, and mixtures of two or more of them.

[0013] In accordance with a preferred embodiment, the production process of the present invention comprises the steps of preparing a solution by dissolving a DM hydrochloride powder having a relative purity of greater than 90% in methanol (for example, by using the DM hydrochloride powder and methanol in a weight ratio of 1 : 5 to 1 : 20); and crystallizing the DM by adding 1-butanol or a mixture of 1-butanol and acetone, hexane or diisopropyl ether (for example, containing up to 60% of acetone, hexane or diisopropyl ether) to the aforesaid solution in an amount of about 1 to 20 parts by volume as based on the methanol.

[0014] When the expression "1-butanol/acetone", for example, is used in connection with the present invention, it means the combined use of 1-butanol and acetone. Thus, according to the present invention, the solvent used to dissolve a DM hydrochloride powder may comprise not only methanol alone, but also a mixture of methanol and 1-butanol or a mixture of methanol, 1-butanol and acetone, hexane or diisopropyl ether, provided that the mixture can dissolve the DM hydrochloride powder. Then, as a solvent for crystallization purposes, 1-butanol or a mixture of 1-butanol and acetone, hexane or diisopropyl ether is added to the DM hydrochloride solution thus obtained, so that a crystalline DM hydrochloride is formed. This crystallization step may be carried out by, after the addition of the aforesaid solvent for crystallization purposes, allowing the solution to stand at a temperature of about 5 to 35°C and preferably at room temperature (18 to 27°C), optionally with cooling (to about 5°C) and optionally with gentle stirring. The crystalline DM hydrochloride so precipitated may be collected by a per se known technique such as filtration or centrifugation.

[0015] DM hydrochloride may be obtained as a commercial product, or may be prepared according to the process described in Japanese Patent Laid-Open No. 21394/'84 (corresponding to U.S. Patent No. 4,592,999). As the starting material for use in the process of the present invention, the DM hydrochloride which has been obtained by any method may be used, provided that it suits the purpose of the present invention. However, it is generally favorable to use DM hydrochloride having a purity of not less than 90% and preferably not less than 95%.

[0016] The present invention is more specifically explained with reference to the following examples. However, it is not intended to limit the present invention to any of these examples.

Example 1 (comparative example)

**[0017]** 2 g of DM hydrochloride was dissolved in 20 mL of methanol. At room temperature, 200 mL of acetone was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 1.2 g of a reddish-brown powder. The result of measurement of this powder according to the X-ray powder diffraction method is shown as a) in FIG. 1. The measuring conditions included a step angle of 0.02°, a counting time of 1.0 second, a tube voltage of 40.0 kV, and a tube current of 20.0 mA (the same shall apply hereinafter).

Example 2 (the present invention)

**[0018]** 2 g of DM hydrochloride was dissolved in 20 mL of methanol. At room temperature, 200 mL of 1-butanol was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 1.4 g of a reddish-brown crystalline powder. The result of measurement of this powder according to the X-ray powder diffraction method is shown as b) in FIG. 1.

Example 3 (comparative example)

**[0019]** 2 g of DM hydrochloride was dissolved in 20 mL of methanol. At room temperature, 200 mL of ethanol was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 0.9 g of a reddish-brown crystalline powder. The result of measurement of this powder according to the X-ray powder diffraction method is shown as c) in FIG. 1.

Example 4 (comparative example)

**[0020]** 2 g of DM hydrochloride was dissolved in 20 mL of methanol. At room temperature, 200 mL of diethyl ether was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 1.5 g of a reddish-brown powder. The result of measurement of this powder according to the X-ray powder diffraction method is shown as d) in FIG. 1.

Example 5 (comparative example)

**[0021]** 2 g of DM hydrochloride was dissolved in 20 mL of methanol. At room temperature, 200 mL of 1-propanol was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 0.9 g of a reddish-brown powder. The result of measurement of this powder according to the X-ray powder diffraction method is shown as e) in FIG. 1.

Example 6 (comparative example)

**[0022]** 2 g of DM hydrochloride was dissolved in 20 mL of methanol. At room temperature, 200 mL of 2-propanol was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 1.3 g of a reddish-brown powder. The result of measurement of this powder according to the X-ray powder diffraction method is shown as f) in FIG. 1.

Example 7 (comparative example)

**[0023]** 2 g of DM hydrochloride was dissolved in 20 mL of methanol. At room temperature, 200 mL of n-hexane was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 1.3 g of a reddish-brown crystalline powder. The result of measurement of this powder according to the X-ray powder diffraction method is shown as g) in FIG. 1.

Example 8 (comparative example)

**[0024]** 2 g of DM hydrochloride was dissolved in 20 mL of methanol. At room temperature, 200 mL of isopropyl ether was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 1.6 g of a reddish-brown crystalline powder. The result of measurement of this powder according to the X-ray powder diffraction method is shown as h) in FIG. 1.

Example 9 (the present invention)

**[0025]** 0.5 g of DM hydrochloride was dissolved in 5 mL of methanol. At room temperature, 50 mL of a mixture (2 : 3) of 1-butanol and acetone was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 0.28 g of a reddish-brown crystalline powder. The result of measurement of this powder according to the X-ray powder diffraction method showed the same pattern as that of b) in FIG. 1.

Example 10 (the present invention)

**[0026]** 0.5 g of DM hydrochloride was dissolved in 5 mL of methanol. At room temperature, 50 mL of a mixture (3 : 2) of 1-butanol and hexane was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 0.38 g of a reddish-brown crystalline powder. The result of measurement of this powder according to the X-ray powder diffraction method showed the same pattern as that of b) in FIG. 1.

Example 11 (the present invention)

**[0027]** 0.5 g of DM hydrochloride was dissolved in 5 mL of methanol. At room temperature, 50 mL of a mixture (3 : 2) of 1-butanol and diisopropyl ether was added to the solution, resulting in the formation of a precipitate. This precipitate was collected by filtration and dried (under reduced pressure at 60°C for 16 hours) to obtain 0.38 g of a reddish-brown crystalline powder. The result of measurement of this powder according to the X-ray powder diffraction method showed the same pattern as that of b) in FIG. 1.

Example 12 (tests for hygroscopicity)

**[0028]** Samples of the powders (or crystalline powders) obtained in Examples 1-8 were stored at 30°C and at relative humidities ranging from 32 to 91%. Their moisture contents were measured until a steady state was reached. The critical relative humidities calculated from the increases or decreases in moisture content are shown in Table 1 below.

Table 1

| Powder | Ex.1 | Ex.2 | Ex.3 | Ex.4 | Ex.5 | Ex.6 | Ex.7 | Ex.8 |
|---|---|---|---|---|---|---|---|---|
| Critical relative humidity (%) | 34 | 73 | 41 | 28 | 29 | 29 | 41 | 53 |

**[0029]** It can be seen from the above-described results that the crystalline DM hydrochloride obtained in Example 2 (the present invention) has very low hygroscopicity.

Example 13 (tests for chemical stability)

**[0030]** Each of the same samples as used in Example 12 was placed in a hermetically sealed container and stored at 60°C for 1 month. Then, the sample was analyzed by HPLC to determine the DM content in the sample. The results thus obtained are shown in Table 2 below.

Table 2

| Powder | Ex.1 | Ex.2 | Ex.3 | Ex.4 | Ex.5 | Ex.6 | Ex.7 | Ex.8 |
|---|---|---|---|---|---|---|---|---|
| Amount of remaining DM (%) | 91.4 | 100 | 97.1 | 90.4 | 97.5 | 94.2 | 97.2 | 96.5 |

**[0031]** It can be seen from the above-described results that the crystalline powder of Example 2 has excellent chemical stability.

(Conditions for analysis by HPLC)

[0032]

Column: YMC A-312 (ODS) (manufactured by YMC Co., Ltd.).
Mobile phase: Acetonitrile-water (38 : 62) (adjusted to pH 2.2 with phosphoric acid).
Flow velocity: About 1.5 ml/min.
Detection: 254 nm.

Example 14 (residual solvent content)

[0033] Each of the same samples as used in Example 12 was analyzed by gas chromatography (GC) to determine its residual solvent content. The results thus obtained are shown in Table 2 below.

Table 3

| Crystalline form | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Powder | Ex.1 | Ex.2 | Ex.3 | Ex.4 | Ex.5 | Ex.6 | Ex.7 | Ex.8 |
| Residual solvent content (%) | 0.14 | 0.40 | 0.03 | 0.50 | 0.19 | 0.18 | 0.05 | 0.95 |

[0034] It can be seen from the above-described results that the residual solvent content of the crystalline powder of Example 2 is within an acceptable limit.

(Operating conditions for analysis by GC)

[0035]

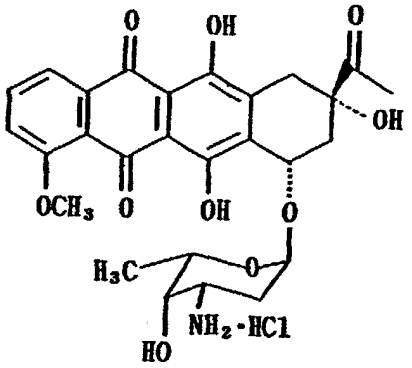
Detector: Flame ionization detector.
Column: Shimadzu CBP 10-S25-050.
Column temperature: Operated at 40°C for 5 minutes, and then raised to 80°C in 5 minutes and held at that temperature.
Vaporization chamber temperature: A constant temperature around 200°.
Carrier gas: Helium.
Flow rate: A constant flow rate at which the retention time of an internal standard substance (dioxane) is about 6 minutes.

## Exploitability in Industry

[0036] The present invention provides crystalline forms of DM hydrochloride showing a reduction in hygroscopicity and residual solvent content and an improvement in chemical stability, as well as a process which can produce them easily. Accordingly, the present invention may be utilized, for example, in the field of the manufacture of medicines and bulk materials for medicines.

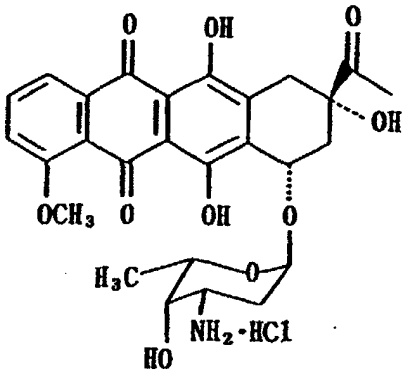
## Claims

1. A crystalline form of anthracycline antibiotic represented by the following formula (I) and having at least characteristic 2θ values (in degrees) of 6.18, 7.88, 9.82, 11.60, 13.30, 15.80, 20.88 and 23.12 as measured by the X-ray powder diffraction method.



(I)

2. A process for producing a crystalline form of anthracycline antibiotic represented by the following formula (I) and having at least characteristic 2θ values (in degrees) of 6.18, 7.88, 9.82, 11.60, 13.30, 15.80, 20.88 and 23.12 as measured by the X-ray powder diffraction method,



(I)

from a solution containing the antibiotic, the process comprising the steps of preparing said solution by using a solvent system composed of a poor solvent for the antibiotic and a good solvent which is miscible with the poor solvent and capable of dissolving the antibiotic; and subjecting the solution so prepared to a crystallization treatment.

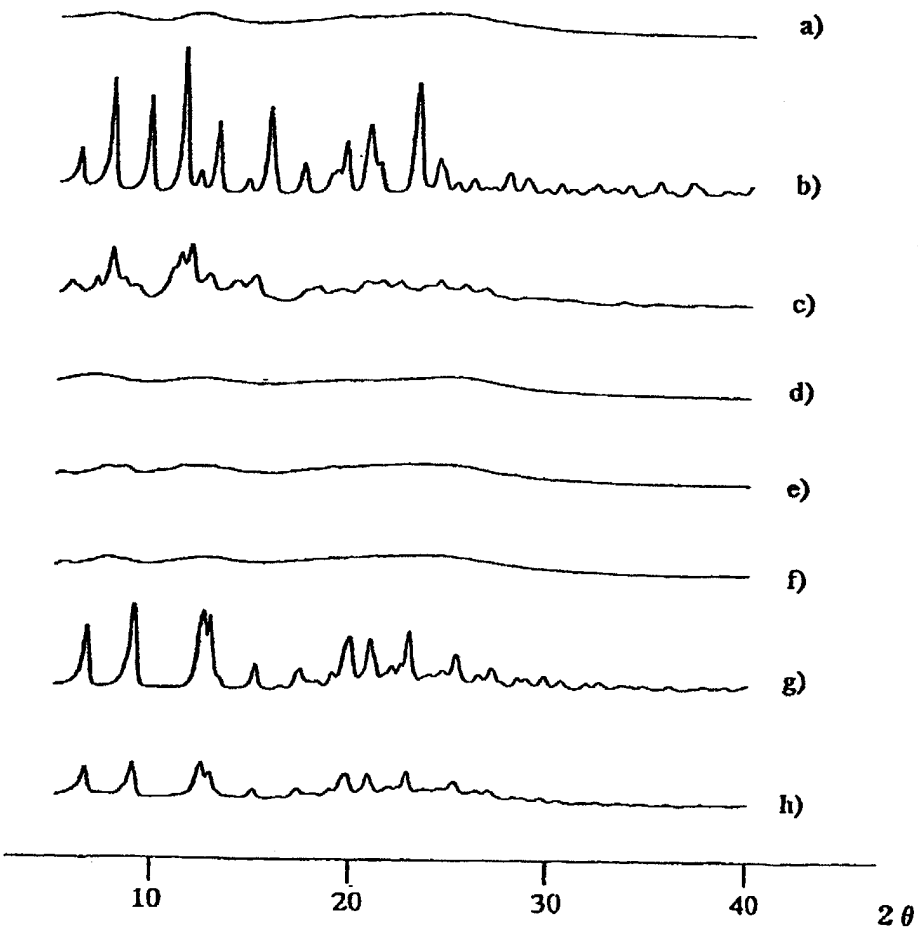
3. A process as claimed in claim 2 wherein the poor solvent contains at least 1-butanol.

4. A process as claimed in claim 2 wherein the poor solvent is selected from the group consisting of 1-butanol, 1-butanol/acetone, 1-butanol/hexane and 1-butanol/diisopropyl ether.

5. A process as claimed in claim 2 wherein the poor solvent is selected from the group consisting of 1-butanol, 1-butanol/acetone, 1-butanol/hexane and 1-butanol/diisopropyl ether, and the good solvent capable of dissolving the antibiotic and used in combination with the poor solvent is selected from the group consisting of water, methanol, ethanol and a mixture of two or more of them.

6. A process as claimed in claim 2 which comprises the steps of dissolving 1 part by weight of the antibiotic of formula (I) in 5 to 20 parts by weight of methanol, adding 1-butanol or a solvent mixture comprising 1-butanol/acetone, 1-butanol/hexane or 1-butanol/diisopropyl ether (in which acetone, hexane or diisopropyl ether may comprise up to 60% by volume of the solvent mixture) to the resulting solution in an amount of 1 to 20 parts by volume based on the volume of methanol, and crystallizing the antibiotic at a temperature in the range of 5 to 35°C.

Fig. 1



# INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP98/05391

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[6] C07H15/252

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[6] C07H15/252

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAplus (STN), REGISTRY (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | JP, 5-399, B2 (Biogal Gyogyszerggyar), 5 January, 1993 (05. 01. 93) & EP, 306541, A & AU, 8775669, A & DK, 8703560, A & FI, 8703062, A & DE, 3775928, G & ES, 2027662, T3 | 1-6 |
| A | JP, 59-118797, A (Farmitalia Carlo Erba S.p.A..), 9 July, 1984 (09. 07. 84), Refer to Example 2 & BE, 898506, A & DE, 3345445, A & GB, 2133005, A & FR, 2538394, A & NL, 8304327, A & SE, 8307134, A & AU, 8322455, A & DK, 8305940, A & FI, 8304650, A & PT, 77883, A & HU, 35270, T & ES, 8504758, A & CA, 1204738, A & CH, 657623, A & AT, 8304400, A & IT, 1155446, B & US, 4861870, A & KR, 9200102, B1 | 1-6 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"E" earlier document but published on or after the international filing date
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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 8 February, 1999 (08. 02. 99) | 16 February, 1999 (16. 02. 99) |
| Name and mailing address of the ISA/ Japanese Patent Office | Authorized officer |
| Facsimile No. | Telephone No. |

Form PCT/ISA/210 (second sheet) (July 1992)

Corresponding to EP 4861875

D2

⑲ 日本国特許庁（JP）　　⑪ 特許出願公開

⑫ 公開特許公報（A）　　昭59—118797

⑤1Int. Cl.³　識別記号　庁内整理番号　⑬公開　昭和59年（1984）7月9日
C 07 H 15/24　7252—4C

発明の数　1
審査請求　未請求

（全 5 頁）

⑤4アントラサイクリノングリコシドの精製法

⑳特　願　昭58—241118
㉒出　願　昭58(1983)12月22日
優先権主張　㉜1982年12月23日㉝イタリア（ＩＴ）㉛24939Ａ/82

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明　細　書

1. 発明の名称　アントラサイクリノングリコシドの精製法

2. 特許請求の範囲

1）粗生成物の水溶液を樹脂上に選択的に吸着させることにより精製を実施することからなるアントラサイクリノングリコシドの精製法。

2）精製すべき粗生成物を含有する溶液を樹脂上に吸着させる前にpH3～5となすことからなる前記特許請求の範囲第1項記載の方法。

3）吸着に使用される樹脂が重合体状イオン交換吸着樹脂およびカルボキシメチルセルロースからなる群から選択されることからなる前記特許請求の範囲第1項記載の方法。

4）精製すべき粗生成物を含有する溶液の樹脂への吸着がただ1種の型の樹脂を用いることにより、または不純物の性質に応じて異なる型の樹脂を適当に選択された順序で用いることにより実施されることからなる前記特許請求の範囲第1項記載の方法。

5）粗製された物質を樹脂から脱着することからなる前記特許請求の範囲第1項記載の方法。

6）樹脂からの精製された物質の脱着が弱酸性の水、または水と極性溶媒との混合物を用いて溶離することにより実施されることからなる前記特許請求の範囲第5項記載の方法。

7）樹脂上への選択的吸着による精製が発酵により得られたアントラサイクリノングリコシドおよび同様の合成化合物のいずれにも使用されうることからなる前記特許請求の範囲第1項記載の方法。

3. 発明の詳細な説明

本発明は樹脂上への選択的吸着によるアントラサイクリノングリコシドの精製法に関する。

前記グリコシドを実質上純粋な形態で取得するのに使用するための高度に特異的な方法を得る必要性は、発酵的製造法および合成的製造法のいずれにおいても粗生成物中における有機および無機不純物の量が特に高くそして平均12～25%まで迄するという事実から主として生ずる。

しかしながら、塩素化された溶媒での粗生成物の抽出そして次に緩衝液での洗浄を行なう従来既知の精製操作による方法では、不純物%が相当低下しているにしてもなお4.5～5.0%の範囲にある最終生成物を生ずる。

一方では一般的に受容しうるものと考えられるが、前記の純度標準は配糖体分子の取扱いおよび化学的試薬に対する特別な感受性を考慮すると、実質上純粋な最終生成物の取得を可能にする改良された精製法の研究における興味を刺激した。抗腫瘍剤としてのアントラサイクリノングリコシドの臨床的使用、そして薬用量および毒性という副次的問題を考慮しつつ、一般的に毒性の高い不純物の最適な除去を達成できることは本発明の明らかな有用性である。

不純物を最高2%の量で含有する最終生成物の取得を可能にする本発明の方法は粗生成物を酸性環境(pH 3～5)で種々のタイプの樹脂上に吸着させそして次に水または水と極性溶媒との混合物を用いて溶離する方法に実質的に基いている。より詳しくは、本発明方法は水溶性物質を溶解含有する弱酸性水性媒体を顆粒またはビードのような粒子中に分散された樹脂上に吸着させる段階および第1の段階において吸着された物質を脱着させる引続く段階を包含するものである。

吸着する樹脂は、実質上一般的に好都合には樹脂粒子を充填した塔またはカラムの形状をした適当な容器中に含有されうる。粗生成物中に含有される不純物の種類の如何により、最初の樹脂からの物質の脱着により得られる溶出液は好都合には引続いて異なる型の樹脂上に吸着され、そこから次に既知方法で溶離されうる。

本発明のクロマトグラフイ精製法においては、重合体状且つイオン交換型またはカルボキシメチルセルロース型の吸着樹脂が使用された。精製すべき粗生成物に応じた種々の型の樹脂の使用における正しい選択および適当な順序そして吸着期間中の弱い酸性環境により、高純度の最終生成物の取得、塩素化有機溶媒使用の排除および優れた精製収率が得られる。

以下の非限定的例により本発明方法を説明する。

例　1

4'-デメトキシダウノルビシンの精製

力価70.2%および不純物含量18%を有する精製状態の4'-デメトキシ-ダウノルビシン15.0gを0.5%酢酸ナトリウム溶液3.6l中に溶解させる。酢酸の添加によりpH 4.7としたこの溶液を直径2.5cmのカラム中のアンバーライト(Amberlite®)XAD2型樹脂〔ローム・アンド・ハース社製品〕400ml上に吸着させる。生成物を水1000mlで洗いそして次に水/メタノール(5:1)(v/v)混合物を用いて溶離する。第1番目の溶出液として、アグリコンおよび種々の不純物を含有する溶液2000mlを集める。

水/メタノール(1:1)(v/v)混合物を用いて溶離を続けそして4500mlのフラクションを集める。溶液状態において溶出液は前記4'-デメトキシ-ダウノルビシンを10%の不純物と共に含有する。

この溶出液を塩酸の添加によりpH2.8となしそして次に真空下に濃縮して容量1500mlとなす。濃縮された溶液に酢酸ナトリウムを添加することによりpHを4.0となしそしてこの弱酸性溶液を直径2.5cmのカラム中に含有されたCMセファロース(Sepharose®)C1.6B型樹脂〔ファルマシア社製品〕150ml上に流量毎時150mlで吸着させる。吸着完結後、カラムを初め水450mlでそして次に0.03%塩酸で溶離する。

不純物18%を含有する初めの溶出液800mlを酢酸ナトリウムの添加によりpH4.7としそして第1番目の精製段階のアンバーライトXAD2樹脂上に吸着させるべき粗製4-デメトキシ-ダウノルビシンの溶液に加えて再循環させる。

純粋な4-デメトキシ-ダウノルビシンを含有する続く溶出液(3500ml)を容量50mlとなるまで真空濃縮する。アセトン250mlを加え、得られた沈殿を戸過し、アセトンで洗いそして乾燥する。出発粗生成物に基いて計算して52%の収率で4-デメトキシ-ダウノルビシンが力価97%および不純物含量3%以下で得られる。

例 2

ダウノルビシンの精製

力価74.2%および不純物含量8.5%を有する塩素化ダウノルビシン15.0gを水4500ml中に溶解させる。酢酸ナトリウムの添加によりこの溶液をpH5.0となしそして直径2.5cmのカラム中のS112カステル(Kastell®)樹脂またはアンバーライトER180型樹脂(ローム・アンド・ハース社製品)400ml上に流量毎時600ml(1.5b.v)で吸着させる。

この生成物を1%塩化ナトリウム溶液1000mlで洗いそして次に水/エタノール(1:1)(v/v)混合物で溶離する。

溶液中にアグリコンを含有する最初のフラクション(400ml)を除去しそして溶離を継続して純粋なダウノルビシンを含有する溶出液260mlを集める。希塩酸の添加によりpHを2.5に調整し、アセトンを添加しそして生成物を+5℃で6時間結晶化せしめる。生成物を戸過し、アセトンで洗いそして12時間真空乾燥する。

出発粗生成物に基いて計算して収率80%でダウノルビシンが得られる。HPLCにより測定して力価97%、不純物含量2.6%である。

例 3

4-デメトキシ-ドキソルビシンの精製

不純物含量14%を有する4-デメトキシ-ドキソルビシン10.4gを含有する水溶液2000mlを直径2.5cmのカラム内に含有されるER180型樹脂(ローム・アンド・ハース社製品)50ml上に流量毎時250mlで吸着させる。

不純物の選択的吸着により部分的に精製された溶出液を次にCMセファロースC1.6B型樹脂200ml上に吸着させる。生成物をメタノール/水/濃塩酸(50:50:0.015)の混合物を用いて溶離し、アグリコンおよび他の不純物を含有する最初のフラクション(約800ml)を捨てる。次に純粋な物質を含有する溶出液2000mlを集め、そして60mlとなるまで真空濃縮する。アセトン300mlを添加し、3時間撹拌する。沈殿を戸過し、アセトンで洗いそして乾燥する。不純物含量3%の純粋な4-デメトキシ-ドキソルビシン6.5gが理論上の58.2%の収率で得られる。

例 4

4'-デスオキシ-ドキソルビシンの精製

力価69.2%そして有機不純物含量12%ならびに鉱物塩不純物12%を有する4'-デスオキ

シードキソルビシン15.0gを水2000mℓ中に溶解しそしてCMセファデックスC25型樹脂300mℓ上に流量毎時600mℓで吸着させる。吸着が完了すると、溶液を0.03%塩酸で溶離しそして4'-デスオキシ-ドキソルビシンを含有するフラクション(6500mℓ)を得る。

前記溶出液を水酸化ナトリウム溶液の添加によりpH3.8となし、そしてこれをS112カステル樹脂200mℓ上に毎時400mℓの流量で吸着させる。生成物を水600mℓで洗いそして次に塩酸の添加によりpH2.0に酸性化したメタノールで溶離する。

コア溶出液600mℓを得、これを真空濃縮して60mℓとなす。撹拌しながら、この濃縮した溶液をアセトン600mℓ中に徐々に加える。形成された沈殿を沪過し、アセトンで洗いそして乾燥する。

力価95.8%および不純物含量4.2%を有する4'-デスオキシ-ドキソルビシンが理論上の61%の収率で得られる。

例 5

4'-エピ-ドキソルビシンの精製

力価75.9%および不純物含量15%を有する粗製4'-エピ-ドキソルビシン15.0gを水4000mℓ中に溶解させる。

燐酸ナトリウムの添加によりこの溶液をpH4.8となしそして直径2.5cmのカラム内に含有されるアンバーライトIRC 724型樹脂400mℓ上に毎時1600mℓ(4b.v.)の流量で吸着させる。

カラムを水800mℓで洗いそして次にメタノール/水/濃塩酸(95:5:0.015)の混合物を用いて溶離する。まだ10%の不純物を含有する4'-エピ-ドキソルビシンを含有するコア溶出液4000mℓおよび不純物含量の多い後部溶出液1500mℓを集める。

コア溶出液を1500mℓまで濃縮し、燐酸ナトリウムの添加によりpH4.8となしそして直径25cmのカラム内に含有されるカルボキシメチルセルロース「ワスマン(Wathman®)」樹脂250mℓ上に毎時500mℓ(2b.v.)の流量で吸着させる。

吸着が完了すると、生成物をエタノール/水/濃塩酸(99.3:0.7:0.015)混合物で洗いそして次にエタノール/水/濃塩酸(90:10:0.05)の混合物で溶離してコア溶出液3200mℓを集める。

溶出液を60mℓとなるまで真空濃縮しそしてアセトン300mℓの添加により生成物を沈殿させる。

力価91.2%および不純物含量3%を有する4'-エピ-ドキソルビシン6.9gが得られる。収率55.3%。

例 6

ドキソルビシンの精製

(a) ドキソルビシン70gを水2.8ℓ中に溶解させる。緩衝液の添加によりpH3.7～4.5となしそしてこの溶液を直径6cmを有するカラム内に含有されるS112カステル樹脂2ℓ上に吸着させる。生成物を水35ℓおよびメタノール15ℓの混合物で溶離する。

溶出液(40ℓ)を0.5ℓとなるまで真空濃縮しそして5℃で3時間撹拌しながらエタノール1ℓおよびアセトン4.5ℓの混合物を添加することにより生成物を結晶化させる。

この生成物を沪過し、0.8ℓのアセトンで洗いそして40℃で5時間真空乾燥する。1回精製されたドキソルビシン(I)56.0gが得られる。

(b) 前記方法(a)で得られた1回精製ドキソルビシン(I)80.0gを水24ℓ中に溶解させそして緩

衡液の添加によりpH4.0となす。

この溶液を直径12cmを有しそしてカルボキシメチルセルロースワスマン樹脂16ℓを含有するカラムに吸着させる。

カラム流出液を除去する。水3.2ℓで洗つたのち、生成物を塩酸の添加によりpH2.5となした水55ℓを用いて溶離する。

溶出液46ℓを集めそして真空下に0.6ℓとなるまで濃縮しそしてイソプロパノール／アセトン（1：3）混合物の注意深い添加により生成物を結晶化せしめる。

生成物を戸過し、アセトン1ℓで洗いそして＋40℃で4時間乾燥する。力価98.5%および不純物含量1.5%を有するドキソルビシン65gが得られる。